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**EUROPEAN PATENT APPLICATION**

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(54) **Novel nitrilase gene**

(57) The invention relates to a DNA coding for a polypeptide having nitrilase activity with an amino acid sequence as shown in SEQ ID NO:1. As compared with conventional processes, the hydrolysis of nitriles by a

nitrilase gene cloned by genetic recombination is expected to drastically improve the catalytic ability of microorganisms because they can be engineered to contain multiple copies of the same gene.

**EP 0 780 471 A2**

**D scription**FIELD OF THE INVENTION

The present invention relates to a novel polypeptide with nitrilase activity and a gene coding for the same.

BACKGROUND OF THE INVENTION

It is known that microorganisms or microorganism-derived enzymes can be used as catalysts in hydrolysis of nitrile compounds to produce carboxylic acids such as optically active  $\alpha$ -hydroxy acids (see Japanese Laid-Open Patent Publication Nos. 99495/92, 99496/92, 218385/92(=US 5,223,416, =EP-A-0449648), 84198/90(=US 5,283,193, =EP-B-0348901), 99497/92(=US 5,234,826, =EP-A-0473328), 192189/93(=US 5,326,702, =EP-A-0486289), and 237789/94(=EP-A-0610048)) and amino acids (see Japanese Laid-Open Patent Publication Nos. 317394/89, 117493/91, and 79894/92).

As compared with such conventional processes, the hydrolysis of nitriles by a nitrilase gene cloned by genetic recombination is expected to drastically improve the catalytic ability of microorganisms because they can be engineered to contain multiple copies of the same gene.

SUMMARY OF THE INVENTION

For the purpose of preparing a bacterial catalyst with higher catalytic activity, the present inventors cloned a nitrilase gene from Gordona terrae MA-1 to complete the present invention.

That is, the present invention relates to a novel polypeptide with nitrilase activity and DNA coding for the same. Further, this DNA may be its analog derived from homology in genetic code.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a restriction enzyme map of pMA001 where the thick line indicates vector pUC118 and the thin line indicates the region of the MA-1-derived DNA. The arrow in the region of the MA-1 DNA indicates the location and direction of the nitrilase gene found in the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Hereinafter, the present invention is described in detail. The present invention is practiced in the following steps.

(1) Preparation of chromosomal DNA from Gordona terrae MA-1

Chromosomal DNA is isolated and prepared from MA-1.

## (2) Preparation of a probe

Two kinds of synthetic DNA corresponding to partial sequences highly homologous among various nitrilases are prepared and used as primers in polymerase chain reaction (PCR) with the chromosomal DNA from MA-1 as a template whereby a part of a nitrilase gene is amplified. The DNA fragment thus amplified is used as a probe.

## (3) Preparation of DNA Library

The chromosomal DNA is cleaved with restriction enzymes and the resulting fragments are inserted into plasmid vector pUC119 to give a library.

## (4) Preparation of transformants and selection for recombinant DNA

The recombinant DNA library prepared in step (3) is used to transform Escherichia coli and the transformant is screened by colony hybridization using the DNA fragment obtained in step (2) as a probe to identify a transformant containing the target recombinant DNA.

## (5) Preparation of a recombinant plasmid

A plasmid is prepared from the recombinant obtained in step (4).

## (6) Preparation of a restriction enzyme map and identification of the region of the nitrilase gene

A restriction enzyme map of the plasmid obtained in step (5) is prepared and the region (of the nitrilase gene) with which the probe is hybridized is identified.

## (7) Nucleotide sequencing

The nucleotide sequence in the vicinity of the region identified in step (6) is determined.

The MA-1 strain has been deposited as Gordona terrae MA-1 (FERM BP-4535), and plasmid pMA001 containing the nitrilase gene has been deposited as transformant E. coli JM109/pMA001 (FERM BP-5547) containing the said gene, with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan.

EFFECT OF THE INVENTION

As compared with conventional processes, the hydrolysis of nitriles by a nitrilase gene cloned by genetic recombination is expected to drastically improve the catalytic ability of microorganisms because they can be engineered to contain multiple copies of the same gene.

PREFERRED EMBODIMENTS OF THE INVENTION

The present invention is illustrated in detail by reference to the following examples, which however are not intended to limit the scope of the invention.

## Example 1

(1) Preparation of chromosomal DNA from Gordona terrae MA-1

MA-1 was incubated at 30°C for 72 hours under shaking in 100 ml MY medium (0.5 % polypeptone, 0.3 % Bacto yeast extract, 0.3 % Bacto malt extract, 1 % glucose). The cells were harvested and the pellet was suspended in 4 ml saline-EDTA solution (0.1 M EDTA, 0.15 M NaCl (pH 8.0)). 8 mg lysozyme was added to the suspension. The suspension was incubated at 37°C for 1 to 2 hours and then frozen. 10 ml Tris-SDS solution (1 % SDS, 0.1 M NaCl, 0.1 M Tris (pH 9.0)) was added to it under gentle shaking, then 0.1 mg proteinase K (a product of Merk) was added to it and the mixture was incubated at 37 °C for 1 hour. After an equal volume of TE-saturated phenol (TE: 10 mM Tris, 1 mM EDTA (pH 8.0)) was added, it was stirred and centrifuged. A 2-fold excess amount of ethanol was added to the supernatant. DNA thus precipitated was recovered with a glass rod, and the DNA was rinsed with 90 %, 80 %, and 70 % ethanol in this order to wash off the phenol. Then, the DNA was dissolved in 3 ml TE. A solution of RNase A (previously treated at 100 °C for 15 minutes) was added to it to a final concentration of 10 µg/ml, and the mixture was incubated at 37 °C for 30 minutes. After proteinase K was added, the sample was incubated at 37°C for 30 minutes. An equal amount of TE-saturated phenol was added to it, then the mixture was separated by centrifugation into upper and lower layers. The upper layer was subjected twice to the same procedure as above and extracted with an equal amount of chloroform containing 4 % isoamyl alcohol in the same manner (these procedures are referred to hereinafter as phenol treatment). Then, a 2-fold excess amount of ethanol was added to it, and the DNA precipitated was recovered with a glass rod whereby the chromosomal DNA was obtained.

## (2) Preparation of a probe

10µl of the chromosomal DNA (20-fold dilution) obtained in step (1), 10µl reaction buffer (10×), 3µl of 50 mM MgCl<sub>2</sub>, 2 µl of 10 mM dNTP, 1µl (100 pmol) each of an oligonucleotide as shown in SEQ ID NO: 4 as primer #1 and an oligonucleotide as shown in SEQ ID NO:5 as primer #2, and 1 µl Taq DNA polymerase (a product of GIBCO BRL) were mixed and the volume was brought up to 100 µl. The primers were constructed on the basis of amino acid sequences highly homologous among known nitrilases. This solution was incubated at 93°C for 30 seconds (denaturation step), 55 °C for 30 seconds (annealing step) and 72 °C for 2 minutes (elongation step), and this cycle was repeated 30 times. After the reaction was finished, the amplification product was extracted 3 times with chloroform and precipitated with ethanol. The DNA thus recovered was separated by electrophoresis on 0.7 % agarose gel whereby an about 500 bp (450 to 550 bp) DNA fragment considered to code for the MA-1 nitrilase gene was obtained. The DNA fragment thus obtained was labeled using DIG DNA Labeling Kit (Boehringer Mannheim) and used as a probe.

## (3) Preparation of DNA library

50µl of the chromosomal DNA from MA-1 was allowed to react at 37 °C for 10 to 20 minutes with a mixture of 10µl reaction buffer (10 ×), 37 µl sterilized water and 0.5µl restriction enzyme Sau 3AI, and DNA was recovered by ethanol precipitation. It was separated by agarose gel electrophoresis and DNA fragments of 6 kb or more were cut off from the gel and recovered with DNA PREP (K.K. Dia Yatoron). These DNA fragments were inserted into the Bam HI site of an E. coli vector pUC118 fragment using a ligation kit (Takara Shuzo Co., Ltd.) whereby a

recombinant DNA library was obtained.

The pUC118 fragment used in the ligation was prepared in the following manner.

10 µl of pUC118 was allowed to react at 37°C for 2 hours with a mixture of 10 µl reaction buffer (10×), 77 µl sterilized water and 2 µl restriction enzyme Bam HI, and the fragment was subjected to phenol treatment, then precipitated with ethanol, dried and dissolved in 50 µl sterilized water. It was further allowed to react at 65°C with a mixture of 1 µl alkaline phosphatase (Takara Shuzo Co., Ltd.), 10 µl reaction buffer (10×) and 39 µl sterilized water. The fragment was subjected to phenol treatment, then precipitated with ethanol, dried, and dissolved in sterilized water.

#### (4) Preparation of transformants and selection for recombinant DNA

*E. coli* JM109 was inoculated into 1 ml LB medium (1 % Bacto trypton, 0.5 % Bacto yeast extract, 0.5 % NaCl) and incubated at 37°C for 5 hours. 100 µl of the culture was added to 50 ml SOB medium (2 % Bacto trypton, 0.5 % Bacto yeast extract, 10 mM NaCl, 2.5 mM KCl, 1 mM MgSO<sub>4</sub>, 1 mM MgCl<sub>2</sub>) and cultured at 18°C for 20 hours. The cells were harvested by centrifugation, and 13 ml of cold TF solution (20 mM PIPES-KOH (pH 6.0), 200 mM KCl, 10 mM CaCl<sub>2</sub>, 40 mM MnCl<sub>2</sub>) was added to the pellet. The pellet was allowed to stand at 0 °C for 10 minutes and centrifuged again. The supernatant was removed and the precipitate of *E. coli* was suspended in 3.2 ml of cold TF solution. 0.22 ml dimethyl sulfoxide was added to the suspension and it was allowed to stand at 0°C for 10 minutes. 10 µl of the recombinant plasmids (DNA library) obtained in step (3) was added to 200 µl of the competent cells thus prepared. The mixture was allowed to stand at 0°C for 30 minutes, then heat-shocked at 42°C for 30 seconds and cooled at 0 °C for 2 minutes, followed by addition of 0.8 ml SOC medium (2 % Bacto trypton, 0.5 % Bacto yeast extract, 20 mM glucose, 10 mM NaCl, 2.5 mM KCl, 1 mM MgSO<sub>4</sub>, 1 mM MgCl<sub>2</sub>). The mixture was incubated at 37 °C for 60 minutes with shaking. The culture, 200 µl per plate, was plated on an LB agar plate containing 100 µg/ml ampicillin. The plate was incubated at 37 °C. To select a transformant having a nitrilase gene, the colonies grown on the plate were subjected to colony hybridization as follows: The colonies grown on the plate were transferred to a nylon membrane (Biodain A produced by Nippon Paul K.K.) and the microorganisms were lysed. The DNA was fixed on the membrane and then subjected to hybridization with the probe (about 500 kb fragment) obtained in step (2). A transformant having the target recombinant DNA was selected using DIG Luminescent Detection Kit (Boehringer Mannheim).

#### (5) Preparation of a recombinant plasmid

The transformant selected in step (4) was cultured at 37°C overnight in 100 ml LB medium, then collected and washed with sterilized water. 5 ml of solution I (2 mM glucose, 10 mM EDTA, 25 mM Tris-HCl (pH 8.0)) and 25 mg lysozyme were added to the cells. The mixture was allowed to stand at 0 °C for 30 minutes. 10 ml of solution II (1 N NaOH, 5 % SDS) was added to the mixture. The mixture was allowed to stand at 0 °C for 5 minutes. 7.5 ml of solution III (3 M sodium acetate (pH 4.8)) was added to the mixture. The mixture was allowed to stand at 0°C for 30 minutes. The mixture was then centrifuged, then 50 ml ethanol was added to the supernatant, and the supernatant was removed by centrifugation. 5 ml of solution IV (10 mM sodium acetate, 50 mM Tris-HCl (pH 8.0)) and 2.5 µl solution (10 mg/ml) of RNase A were added to it, and it was allowed to stand at room temperature for 20 minutes. 12 ml ethanol was added to it, and the plasmid was recovered by centrifugation and rinsed with 70 % ethanol, dried, and dissolved in 0.4 ml sterilized water. The solution was subjected to phenol treatment and the plasmid was recovered by precipitation with ethanol, dried, and dissolved in 0.4 ml sterilized water. The resulting recombinant plasmid was designated pMA001.

#### (6) Preparation of a restriction enzyme map and identification of the region of the nitrilase gene

The plasmid pMA001 obtained in step (5) was cleaved with several restriction enzymes to prepare its restriction enzyme map (FIG. 1). Separately, pMA001 was cleaved with restriction enzymes such as *Kpn* I, *Xho* I, *Pst* I, *Sal* I etc. and the fragments were subjected to agarose gel electrophoresis. The fragment hybridized with the probe was identified by Southern hybridization.

#### (7) Nucleotide sequencing

The nucleotide sequence in the vicinity of the region identified in step (6) was determined by Fluorescence Sequencer ALF II (Pharmacia). The result indicated the presence of a nucleotide sequence as shown in SEQ ID NO:3, and there was found an open reading frame coding for an amino acid sequence as shown in SEQ ID NO:1. Comparison with amino acid data base NBRF (National Biomedical Research Foundation) revealed that this gene has 30 to 50 % homologies with known nitrilases at the amino acid sequence level, and that this nitrilase also has highly homologous regions in common with known nitrilases, suggesting that this nitrilase is a novel nitrilase. The nucleotide sequence of the open reading frame is shown in SEQ ID NO:2.

Example 2

MA-1 was aerobically cultured at 30 °C for 72 hours in a medium (20 g/l glycerol, 3 g/l yeast extract, 6.8 g/l potassium monophosphate, 7.1 g/l sodium diphosphate, 2.8 g/l sodium sulfate, 0.4 g/l magnesium chloride, 0.04 g/l calcium chloride, 0.03 g/l manganese sulfate, 0.006 g/l iron chloride, 0.003 g/l zinc sulfate, 0.5 g/l benzyl cyanide). The cells were harvested by centrifugation, and the pellet was washed with 50 mM phosphate buffer (pH 8.2) and suspended in the same buffer. The cells were disrupted by sonication and centrifuged to give a supernatant (cell extract). A part of the supernatant was subjected to SDS polyacrylamide gel electrophoresis (SDS-PAGE) and the protein was transferred to PVDF membrane (Immobilon P<sup>SQ</sup> (a product of Millipore)). The membrane was stained with Coomassie Blue, and a band with a molecular weight of about 40 kDa appearing only where an inducer was added to the medium was cut off, and its N-terminal amino acid sequence was analyzed by PSQ-1 Amino Acid Sequence Analyzer manufactured by Shimadzu Corporation. The sequence Thr-Thr-Asp-Tyr-Ser thus obtained agreed with the N-terminal amino acid (excluding N-terminal residue Met) deduced from the nucleotide sequence (SEQ ID NO:2). In addition, analysis of the amino acid sequence suggested that the N-terminal residue Met is not cleaved off and remains.

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## SEQUENCE LISTING

SEQ ID NO: 1:

LENGTH: 344 amino acids

TYPE: amino acid

TOPOLOGY: linear

MOLECULE TYPE: protein

SOURCE

ORGANISM: Gordona terrae

STRAIN: MA-1

SEQUENCE DESCRIPTION: SEQ ID NO: 1:

XaaThrThrAspTyrSerGlyThrPheLysAlaAlaValThrGln	15
AlaGluProValTrpPheAspLeuSerAlaThrValAspLysThr	30
IleAlaLeuValGluGluAlaSerArgAlaGlyAlaAspLeuIle	45
AlaPheProGluThrTrpIleProGlyTyrProTrpPheLeuTrp	60
LeuAspSerValAlaTrpGlnSerGlnTyrPheIleArgTyrPro	75
GlnAsnSerLeuAspLeuAspGlySerGluPheAlaAlaIleArg	90
GluAlaAlaArgLysAsnAspIleAlaIleThrMetGlyPheSer	105
GluArgGlyHisGlySerLeuTyrMetGlyGlnAlaValIleGlu	120
ArgAspGlyValValValArgThrArgArgLysLeuLysProThr	135
HisValGluArgThrLeuPheGlyGluGlyAspGlySerAspLeu	150
ValValAspGlnThrSerLeuGlyArgValGlySerLeuCysCys	165
TrpGluHisLeuGlnProLeuThrLysTyrAlaMetTyrSerGln	180
HisGluGlnIleHisIleAlaAlaTrpProSerPheSerIlePhe	195
ProGlyAlaValTyrAlaLeuGlyProGluValAsnThrAlaAla	210
SerGlnGlnTyrAlaValGluGlyGlnThrTyrValLeuAlaPro	225
CysAlaValIleGlyAspAlaGlyTrpGluAlaPheAlaAspThr	240
GluGluLysArgGlnLeuIleHisLysGlyGlyGlyTyrAlaArg	255
IleTyrGlyProAspGlyArgSerLeuAlaGluProLeuAlaPro	270
AsnAspGluGlyIleLeuTyrAlaAspIleAspLeuSerAlaIle	285

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LeuAlaAlaLysAsnProAlaAspProValGlyHisTyrSerArg 300  
 ProAspValLeuArgLeuGlyPheAsnLysAlaProGlnProLys 315  
 ValAsnIleLeuGlyThrGluProSerArgThrThrSerThrGln 330  
 CysArgProThrThrIleArgArgSerTrpArgPheProGlu 344

(In the sequence, Xaa stands for deletion or Met.)

SEQ ID NO: 2:

LENGTH: 1035 base pairs

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

SOURCE

ORGANISM: Gordona terrae

STRAIN: MA-1

SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATG ACC ACC GAC TAT TCC GGC ACG TTC AAG GCA GCG GTG ACC CAG 45  
 GCC GAA CCG GTG TGG TTC GAC CTC TCG GCC ACC GTC GAC AAG ACC 90  
 ATT GCG CTC GTC GAA GAG GCG TCC CGG GCC GGC GCC GAT CTG ATC 135  
 GCG TTC CCG GAG ACC TGG ATA CCG GGG TAC CCG TGG TTC CTG TGG 180  
 CTT GAC TCG GTG GCC TGG CAG AGC CAG TAC TTC ATC CGG TAT CCG 225  
 CAG AAC TCG CTC GAT CTC GAC GGC AGC GAG TTC GCG GCG ATC AGG 270  
 GAA GCC GCA CGA AAG AAC GAC ATC GCG ATC ACC ATG GGA TTT AGT 315  
 GAG CGC GGT CAT GGT TCG CTG TAC ATG GGC CAG GCG GTC ATC GAG 360  
 CGT GAC GGG GTC GTC GTA CGC ACA CGC CGC AAA CTG AAG CCG ACC 405  
 CAC GTC GAG CGG ACC CTG TTC GGT GAG GGT GAT GGT TCC GAT CTG 450  
 GTC GTG GAC CAG ACC AGT CTC GGC CGA GTC GGG TCG CTG TGC TGT 495  
 TGG GAA CAT CTG CAG CCG TTG ACC AAG TAC GCC ATG TAC TCG CAG 540  
 CAC GAG CAG ATT CAC ATC GCC GCA TGG CCC AGC TTC TCG ATC TTC 585  
 CCG GGC GCG GTG TAT GCG CTC GGG CCC GAG GTC AAC ACC GCG GCC 630

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TCT CAG CAA TAC GCC GTA GAA GGG CAG ACC TAC GTT CTC GCT CCA 675  
 TGC GCG GTC ATC GGC GAT GCA GGT TGG GAG GCG TTT GCC GAT ACC 720  
 GAG GAG AAG CGA CAG CTC ATC CAC AAA GGA GGC GGA TAT GCC CGT 765  
 ATC TAC GGT CCC GAC GGT CGT TCA CTC GCG GAA CCG CTC GCG CCC 810  
 AAT GAC GAG GGA ATC CTG TAC GCG GAC ATC GAT CTG TCT GCG ATT 855  
 CTG GCC GCA AAG AAC CCG GCG GAC CCG GTT GGG CAC TAC TCG CGT 900  
 CCG GAC GTA CTG CGT CTC GGA TTC AAC AAA GCG CCT CAG CCG AAG 945  
 GTC AAC ATC TTG GGA ACG GAG CCG TCT CGG ACG ACG TCG ACG CAG 990  
 TGC CGA CCG ACG ACG ATT CGG AGG TCA TGG CCG TTT CCT GAG TGA 1035

SEQ ID NO: 3:

LENGTH: 1200 base pairs

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

SOURCE

ORGANISM: Gordona terrae

STRAIN: MA-1

SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GTTCAAAAAT AGTCGATCGA TCGTGCTGGC AATGCTACGG ACGTCGGCTG 50  
 TGACGCACCT AACGTTGCTG CCACCAACAA AGAATGGAGT CTCGATGACC 100  
 ACCGACTATT CCGGCACGTT CAAGGCAGCG GTGACCCAGG CCGAACCGGT 150  
 GTGGTTTCGAC CTCTCGGCCA CCGTCGACAA GACCATTGCG CTCGTCGAAG 200  
 AGGCGTCCCG GGCCGGCGCC GATCTGATCG CGTTCCCGGA GACCTGGATA 250  
 CCGGGGTACC CGTGGTTCCT GTGGCTTGAC TCGGTGGCCT GGCAGAGCCA 300  
 GTACTTCATC CCGTATCCGC AGAACTCGCT CGATCTCGAC GGCAGCGAGT 350  
 TCGCGGGCAT CAGGGAAGCC GCACGAAAGA ACGACATCGC GATCACCATG 400  
 GGATTTAGTG AGCGCGGTCA TGGTTCGCTG TACATGGGCC AGGCGGTCAT 450  
 CGAGCGTGAC GGGGTCGTCG TACGCACACG CCGCAAACCTG AAGCCGACCC 500



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ACGTCGAGCG GACCCTGTTT GGTGAGGGTG ATGGTTCCGA TCTGGTCGTG 550  
 GACCAGACCA GTCTCGGCCG AGTCGGGTCTG CTGTGCTGTT GGGAACATCT 600  
 GCAGCCGTTG ACCAAGTACG CCATGTACTC GCAGCACCAG CAGATTCACA 650  
 TCGCCGCATG GCCCAGCTTC TCGATCTTCC CGGGCGCGGT GTATGCGCTC 700  
 GGGCCCGAGG TCAACACCGC GGCCTCTCAG CAATACGCCG TAGAAGGGCA 750  
 GACCTACGTT CTCGCTCCAT GCGCGGTCAT CCGCGATGCA GGTGGGAGG 800  
 CGTTTGCCGA TACCGAGGAG AAGCGACAGC TCATCCACAA AGGAGGCGGA 850  
 TATGCCCCGTA TCTACGGTCC CGACGGTCGT TCACTCGCGG AACCGCTCGC 900  
 GCCCAATGAC GAGGGAATCC TGTACGCGGA CATCGATCTG TCTGCGATTC 950  
 TGGCCGCAAA GAACCCGGCG GACCCGGTTG GGCCTACTC GCGTCCGGAC 1000  
 GTACTGCGTC TCGGATTCAA CAAAGCGCCT CAGCCGAAGG TCAACATCTT 1050  
 GGGAACGGAG CCGTCTCGGA CGACGTCGAC GCAGTGCCGA CCGACGACGA 1100  
 TTCGGAGGTC ATGGCGGTTT CCTGAGTGAC AAGGTGCTGG CGACCGCCGC 1150  
 CGGAATGGCG GAAAATCATC AGTAATGGGC GATTGCGCCA CTCGTGCGCC 1200

SEQ ID NO: 4:

LENGTH: 20 base pairs

TYPE: nucleic acid

SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CGBCGBAARCTSAARCCNAC

(In the sequence, B stands for G, C or T; R for G or A; S for G or C; and N for G, C, A or T.)

SEQ ID NO: 5:

LENGTH: 20 base pairs

TYPE: nucleic acid

SEQUENCE DESCRIPTION: SEQ ID NO: 3:

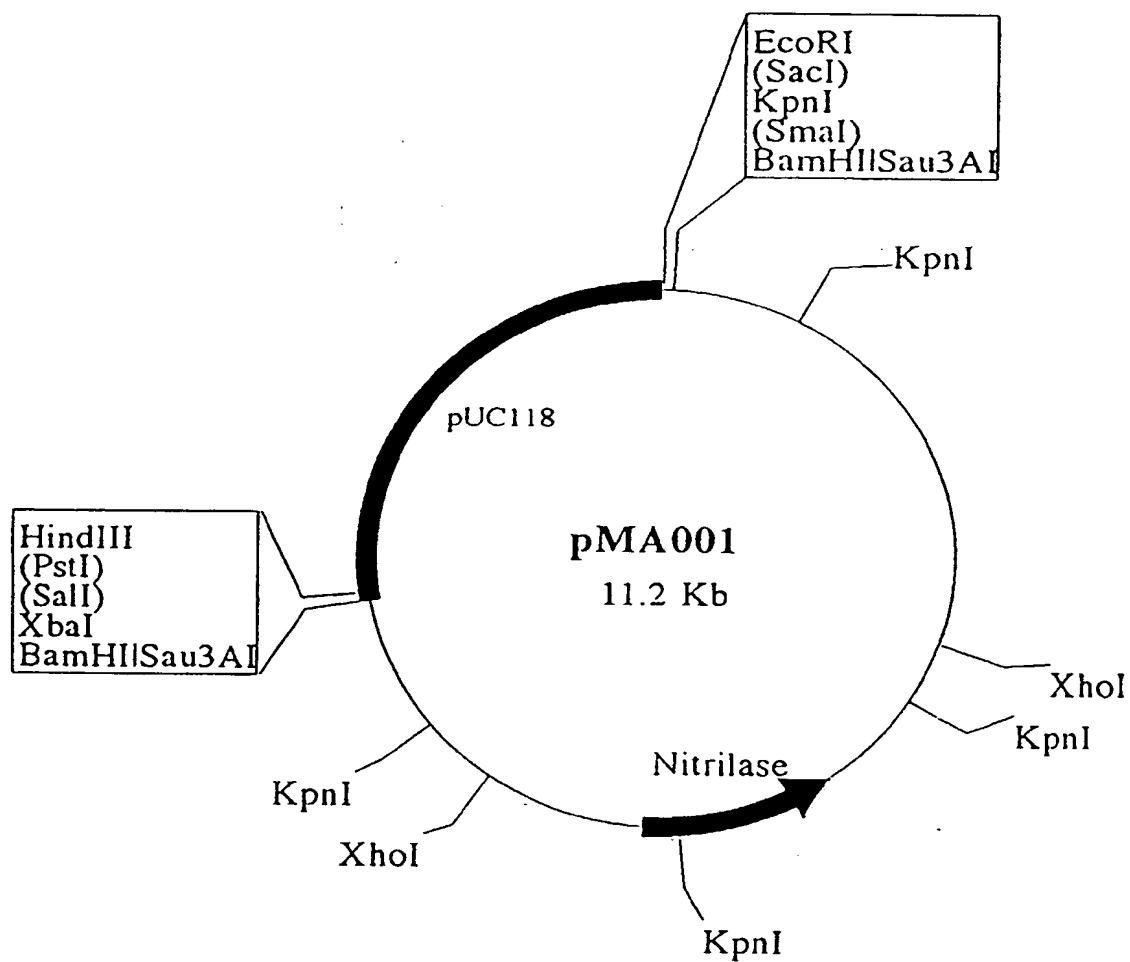
GARTARTGRCCSACVGGRTC

(In the sequence, R stands for G or A; S for G or C; and V for G, C or A.)

Claims

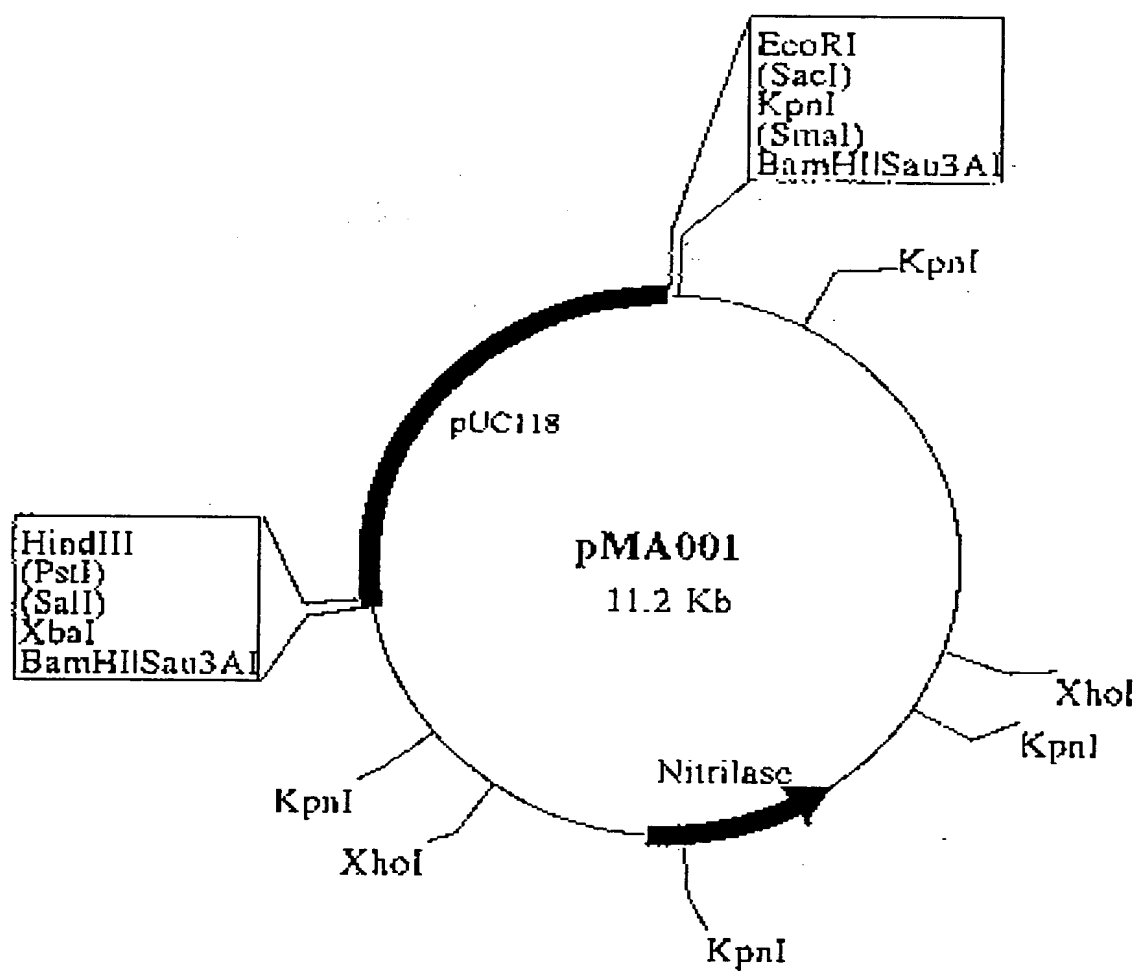
1. A DNA coding for a polypeptide having nitrilase activity with an amino acid sequence as shown in SEQ ID NO:1, or a polypeptide analogue thereof which comprises a homologous sequence.
2. A DNA according to claim 1 which comprises the nucleotide sequence of SEQ ID NO:2.
3. A DNA according to claim 1 which comprises a homologous sequence coding for a polypeptide, said polypeptide having nitrilase activity and a sequence homology at the amino acid level of at least 30 to 50%, preferably at least 70%.
4. A DNA according to claim 3 wherein the homology is at least 80%, preferably at least 90%.
5. A DNA capable of hybridizing to the nucleotide sequence of SEQ ID NO:2 or a fragment thereof which codes for a polypeptide having nitrilase activity.
6. A DNA fragment of 450 to 550 base pairs in size obtainable by amplification of DNA with the primers of SEQ ID NOS:4 and 5.
7. A DNA according to any one of claims 1, 2, 3, 4, 5 or 6 which is an isolated DNA.
8. A DNA expression vector comprising a DNA according to any one of the preceding claims operably linked to a promoter.
9. A host cell comprising one or multiple copies of a DNA according to any one of claims 1 to 8.
10. A process for preparing a nitrilase which comprises culturing a host cell according to claim 9 and optionally recovering said nitrilase.
11. A process for the hydrolysis of a nitrile compound which comprises bringing the nitrile compound into contact with a nitrilase produced by the process of claim 10 under conditions suitable to produce the corresponding carboxylic acid, particularly an  $\alpha$ -hydroxy acid, said process optionally further comprising recovering said carboxylic acid.

FIG. 1



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FIG. 1



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European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number  
EP 96 30 5632

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 267, no. 29, 1992, pages 20746-20751, XP002031260 KOBAYASHI, M. ET AL.: "Nitrilase from Rhodococcus rhodochrous J1" * the whole document *	1,3,6-11	C12N9/78 C12N1/21 C12N15/09 C12N15/55
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
			C12N
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 21 May 1997	Examiner Hillenbrand, G
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application I : document cited for other reasons & : member of the same patent family, corresponding document			

EPO FORM 150 (01.82) (P0401)